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LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NY 10023

EXAMINER	
PANDE, SUCHIRA	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/672,746	LIAO ET AL.	
	Examiner	Art Unit	
	Suchira Pande	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *28 August 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-19* is/are rejected.

7)☒ Claim(s) *1* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28 Aug 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *12/1/2003*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

1. This office action is in response to an amendment filed on August 28, 2006.

Claims 1-15 were previously pending. Applicant amended claims 1-6 and 9-11; and added new claims16-19. Claims 1-19 are currently pending and will be examined. Applicant's amendment overcame the objections to the Drawings, Specification, and Claim 1.

Claims 1-4 and 10-11 were rejected under 102(b). Amendment to claim 1 step (2) "wherein each polymerase chain reaction of the multi-cyclic polymerase chain reactions is conducted on a template that is a product obtained in a previous polymerase chain reaction" obviate the 102(b) rejections of claims 1-4 and 10-11 based on Horton et. al. made in the Office Action of 3 May 2006.

Rejected claims 5-9 and 12-15 depend on claim 1. The 103 rejections were based on Horton et. al. in view of appropriate reference. Since Horton et. al. does not teach all the limitations of claim 1 as enumerated above accordingly 103 rejections of claims 5-9 and 12-15 made in the Office Action of 3 May 2006 are withdrawn.

Drawings

2. The replacement drawing for Fig 7 was received on 28 August 2006. These drawings are acceptable.

Specification

3. The amendments to specification received on 28 August 2006 to correct the informalities are acknowledged and entered.

Claim Objections

4. The objection to Claim 1 cited in Office Action mailed on May 3, 2006 is removed as Applicants have added the missing prime marks.

5. All other previously presented rejections are maintained for the reasons given below.

Claim Objections

6. Amended claim 1 is objected to because of the following informalities: Step (2) contains a typographical error: multi-cyclic polymerase "claim" should read "chain" reactions----Appropriate correction is required.

Claim Rejections - 35 USC § 112

7. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1-19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In the first action on merits Examiner had rejected claims 1-15 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites, "first template is any template sequence commonly used in the host-vector expression system or *a fragment of the target polynucleotide*". Therefore, it is this limitation that creates the confusion, since if the target is a template, then the

claimed primers do not make sense because the same primer cannot have two parts which are identical to the opposite ends of the template.

That means the art cited and interpretation applied by Examiner for template sequence is still correct. Page 64, Figure A of Horton et. al. shows the first template H-$2K^b$ contains only a fragment of the target polynucleotide (fragment CD or fragment GH). The target recombinant molecule AH lacking intron 2 does not exist in nature. So it meets the criteria cited by applicant in page 18 par. 3 of the traversal argument.

Examiner understands the invention with help of the traversal arguments provided by applicant on page 19 where applicant points out the invention with help of the primers used in the right column of Fig. 1. However what renders the claim indefinite is the fact how these primers are claimed.

As Examiner understands the crux of the invention lies in the fact that two part primers can be used to perform primer extensions. The 3' end of the two part primers are anchored while the 5' end of the two-part primer is unanchored. This unanchored part is used to introduce new sequences in each amplification cycle resulting in final product (being referred by applicant as target sequence). If this is the intent, the claims need to be written to clearly reflect the above intent.

Once the template DNA 1 is denatured and the two strands are amplified as depicted in Fig. 1 Right panel using primers 1 and 2 then the 5' end of molecule 1 and the 3' end of the molecule 2 resulting from first primer extension will have added sequences. Thus 50% of the DNA molecules formed at end of step 2 will have additional sequences in their 5' end and 50% of the DNA molecules will have additional

sequences in their 3' end. So now the question arises which species of molecules are being referred to (molecule 1 or molecule 2) with reference to which the 5' and 3' end characteristics of the claimed primers are to be considered. When applicant is referring to template that has been extended using the primers of the current invention then again the question is which of these molecules is being referred to when the 5' and 3' end of the template is being referred to. The same problem continues with the final product (target sequence).

All claims 1-19 under consideration stand rejected under 35 U.S.C. 112, second paragraph as being indefinite. Since the amendments to the claims still do not make the claimed subject matter clear.

9. Currently amended Claims 2 and 3 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The limitation of claim 2 wherein "the first template comprises ***a sequence not found in the target polynucleotide sequence***" is contradicting the wherein clause of claim 1 where "the first template is---------or ***a fragment of the target polynucleotide***". It is not clear how it is possible to have a first template that is consistent with limitations spelled out in claims 1 and 2 simultaneously.

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

12. Claims 1-4, 10-11 and 16-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Horton et. al. (1989) Gene 77:61-68 (cited in previous Office Action) as evidenced by Ho et. al. (1989) Gene 77 51-59 (cited by applicant in IDS) in view of Zhang et. al. (1997) Proc. Natl. Acad. Sci. USA Vol. 94, pp 4504-4509.

Regarding claim 1, Horton et. al. teaches:

1) conducting a first polymerase chain reaction on a first template with a first

primer pair to obtain a first polymerase chain reaction product. (See Horton et. al. page 63, Fig. 1 Gene I or Gene II);

(2) conducting muti-cyclic polymerase chain reactions by a primer extension technique to obtain a product comprising the target polynucleotide sequence; (see Horton et. al. page 63 legend to Fig 1. lines 7-10).

(i) a second set of primer pairs consisting of a forward primer and a reversed primer, the forward primer having two parts:

(a) the part (a1), located at the 5'-end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the fragment at the 3' end region of the target polynucleotide sequence, and

(b) the part (b1), located at the 3' end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the sequence of the more than 10 nucleotides from the 5' end region of the template sequence; and wherein the 3' end of the part (a1) is adjacent to the 5 end of the part (b1); and the reversed primer having, at the 3 end region of the reversed primer, a fragment having more than 5 nucleotides and being capable of annealing to the 3'- end region of the template sequence;

Horton et. al. teach (see primers c and d in Horton et. al. page 63 Fig 1) primer pairs described in (i) of step 2 of claim 1; the forward primer having two parts (see primer c) and reverse primer d. Horton et. al. also teach (see primers a and b in Horton et. al. page 63 Fig 1) primer pairs described in (ii) of step 2 of claim 1; the forward primer a and the reverse primer having two parts (see primer b).

Horton et. al. teaches primers "d" and "e" in the extension overlap. These primers have a region of 15 nucleotides that allows them to hybridize in the region of overlap. (see page 65, section labeled B: primers). The sequence 5' t gcc gcc cgc gct ct 3' of primer "d" allows it to hybridize to 3' end of exon 2 of H-2K^b and by PCR with primer pair "c" it allows the generation of the fragment to CD. Similarly sequence 5' ggc act cac aca ctc cag 3' of primer "e" allows it to hybridize to 5' end of exon 3 of H-2L^d and by PCR with primer pair "f" it allows the generation of the fragment to EF. 3' end of fragment CD and 5' end of fragment EF share the region of homology overlap provided by primers 'd" and "e" described above. Thereby strand 5' CD 3' can serve as primer for strand 5' EF 3' and allow extension of the fragment CD to form CDEF or conversely strand 5' FE 3' can serve as a primer for strand 5' DC 3' and allow extension of the fragment EF to form fragment 5' CDEF 3'.

Horton et. al. teaches use of template in PCR that is the product obtained in the previous PCR. See page 65, section labeled D: PCR products. Here the PCR products fragment "AD" and fragment "EH" serve as templates for the production of recombinant product shown in section E page 65. In this case the 3' end of fragment "AD" shares a region of 15 bp that is homologous to the 5' end of fragment "EH". So the denatured strands of AD and EH can hybridize in the region of overlap to allow for primer extension.

(3) Horton et. al. teaches recovering the polynucleotide product comprising the target polynucleotide sequence from the final product of the multi-cyclic polymerase chain reactions. (The Recombinant Product formed in Horton et. al. page 63 Fig. 1 and

page 64 Fig. 2 B is the target polynucleotide sequence that was not present in the template).

Horton et. al. were interested in creating a chimeric protein containing parts of H2L and H2K so they use these two templates as their starting point (see page 64 Fig. 2.). The example clearly illustrates that the method taught by Horton et. al. can be practiced starting with one single template as envisaged in the present application as the method hinges on the fact that primers have a region of overlap that can be used for extending the PCR product in subsequent cycles.

Horton et. al. make several PCR products that had the overlaps created by the way the primers were designed and extended them in pair wise fashion (see Horton et. al. page 64 Fig. 2 B) by PCR till they got the final product. It should be noted that the same result would have been obtained if they had chosen to first use primers a and b to make fragment AB then decided to extend the sequence by performing PCR after adding primers c and d to the mix. Looking at the sequence of the primers (see Horton et. al. page 65 for listing on primer pairs and the region of homology between them) a, b, c and d in page 65 it is evident that primers b and c share a 15 nucleotide region of complementarity that would allow extension overlap resulting in formation of fragment ABCD during PCR. Since primer e has a region that is complementary to primer d this pair of primers (e and f) would allow fragment ABCD to be extended by extension overlap to create ABCDEF. PCR product ABCDEF in turn would be extended by primers g and h to generate the final product ABCDEFGH. Horton et. al. (See page 63 Fig. 1 legend lines 7-10) explicitly teaches "The intermediates in this reaction are shown

in the hatched box. The end of one strand from each product is capable of hybridizing with the complementary end from the other product. The strands having this overlap at their 3' ends can act as primers for one another and be extended by the polymerase to form the full-length recombinant product". This teaching indicates that Horton et. al. was perfectly aware that primer extension overlap PCR taught by them could also be practiced in the manner of claim 1 using exact same primers.

Regarding claim 2, Horton et. al. do not specifically point out the limitation recited in claim 2, wherein the first template comprises a sequence not found in the target polynucleotide sequence, and wherein the method further comprises a step of removing the nucleotide sequence of the first template from the final product in the step (3) so as to obtain a product consisting of the target polynucleotide sequence. However Horton et. al. refer to Ho et. al. in their publication (see Horton et. al. page 66 par. 2). Ho et. al. evidence the fact that limitation recited in claim 2 is actually taught by Horton et. al.

Regarding claim 2, Ho et. al. teaches wherein the first template comprises a sequence not found in the target polynucleotide sequence, and wherein the method further comprises a step of removing the nucleotide sequence of the first template from the final product in the step (3) so as to obtain a product consisting of the target polynucleotide sequence (see Ho et. al. page 55 par. 1 where expression vector containing wt class-I gene construct is taught as template. The final target is the expression vector containing mutagenized class-I gene. Once the PCR fragment containing the mutagenized class-I gene has been obtained then the expression vector

containing the wt class-I gene is cut by appropriate flanking restriction sites to remove the nucleotide sequence of the wt class-I gene and replaced by mutagenized class-I gene using standard molecular biology techniques known to one of ordinary skill in the art. Thus obtaining a product consisting of the target polynucleotide sequences namely the expression vector containing the mutant class-I gene).

Regarding claim 3, Horton et. al. do not specifically point out the limitation recited in claim 3 namely wherein the first template is designed to have restriction enzyme recognition sites at both ends. However Horton et. al. refer to Ho et. al. in their publication (see Horton et. al. page 66 par. 2). Ho et. al. evidence the fact that limitation recited in claim 2 is actually taught by Horton et. al.

Regarding claim 3, Ho et. al. teaches wherein the first template is designed to have restriction enzyme recognition sites at both ends. (see Ho et. al. page 55 par. 1 and Fig. 3 A. where restriction sites Hind III and Xho I at both ends of first template are taught. Also the expression vector will contain many other restriction sites in its multicloning site polylinker region).

Regarding claim 4, Horton et. al. teaches primer pairs where fragment that has homology to target polynucleotide in each step is 15 nucleotides. See page 65, Fig. 3, Section C, primer "d" and primer "e" they both share a 15 nucleotide region of homology; and Section D where fragment "AD" and fragment "EH" serve as primers that can be extended by extension overlap here too the region of homology between them is 15 nucleotide. Also see the Fig. 3 legend for Part C and Part D.

Regarding claim 4, Ho et. al. explicitly evidences the limitation of claim 4. Ho et. al. teaches wherein the fragment having more than 10 nucleotides used in each step has more than 15 nucleotides. (see Ho et., al. page 55, par. 1 where 35-mer (primer b2) and a 26 mer (primer c2 and c3 are taught that have a 16 nucleotide region of overlap. Thereby Ho et. al. teaches the fragment having more than 10 nucleotides used in each step has more than 15 nucleotides.)

Regarding claim 10, Horton et. al. teaches production of a mutant target polynucleotide containing multiple mutations as compared to the starting first templates. (see page 64 Recombinant Molecule formed in Fig. 2 B starting from H-2L^d and H-2K^b). Here the first templates were H-2L^d and H-2K^b and the final target produced by extension overlap is chimeric fusion polynucleotide containing sequences from H-2L^d and H-2K^b. In this case, the final construct has parts AB and EF that come from H-2L^d while parts CD and GH come from H-2K^b . The intron 2 region of H-2L^d is deleted in the final construct such that exon 2 is directly fused to exon 3. Thus the recombinant target sequence generated contains multiple mutations such as deletions and replacements compared to starting templates.

Regarding claim 11, Horton et. al. teaches primer "a" used to generate fragment AB (see page 64 Fig. 2 top). This primer meets all the criteria recited for helper primer in the claim.

Regarding claim 16, Horton et. al. does not explicitly teach wherein the first polymerase chain reaction is conducted with primers that consist of first primer pair. However Horton et. al. refer to Ho et. al. in their publication (see Horton et. al. page 66

par. 2). Ho et. al. evidence the fact that limitation recited in claim 16 is actually taught by Horton et. al.

Regarding claim 16, Ho et. al. teaches wherein the first polymerase chain reaction is conducted with primers that consist of first primer pair (See Ho et. al. page 55, where first primer pairs external primers located approximately outside of the unique Hind III and XhoI restriction sites 'a' and 'd' are taught).

Regarding claim 17, Horton et. al. teaches first primer pair as described above for claim 1 and also teaches helper primer as described above for claim 11, therefore Horton et. al. inherently teaches wherein the first polymerase chain reaction is conducted with primers that consist of the first primer pair and the helper primer.

Regarding claim 18, Horton et. al. do not explicitly teach wherein the primers of the first primer pair and the primers of the multi-cyclic polymerase chain reactions comprise no more than 36 nucleotides. However Horton et. al. refer to Ho et. al. in their publication (see Horton et. al. page 66 par. 2). Ho et. al. evidence the fact that limitation recited in claim 18 is actually taught by Horton et. al.

Regarding claim 18, Ho et. al. teaches wherein the primers of the first primer pair and the primers of the multi-cyclic polymerase chain reactions comprise no more than 36 nucleotides (see Ho et. al. page 55 where first primer pairs 'a' and 'd' are each 17 nucleotide long and primers of the multicyclic polymerase chain reactions b1, c1, b2, c2 and c3 are all no more than 36 nucleotides.) Thus Ho et. al. teaches wherein the primers of the first primer pair and the primers of the multi-cyclic polymerase chain reactions comprise no more than 36 nucleotides.

Regarding claim 1, Horton et. al. does not teach:

(2) wherein each polymerase chain reaction of the multi-cyclic polymerase chain reactions is conducted on a template that is a product obtained in the previous polymerase chain reaction;

Regarding claim 1, Zhang et. al. teaches a PCR method where the output of one cycle is the input for the next cycle (see Zhang et. al. page 4504 par. 5. Thereby teaching a method wherein each polymerase chain reaction of the multi-cyclic polymerase chain reactions is conducted on a template that is a product obtained in the previous polymerase chain reaction)

It would have been *prima facie* obvious to one of ordinary skill in the art to practice the method of Zhang et. al. in the method of Horton et. al. at the time of the invention. The motivation to do so is provided by both Ho et. al. and Zhang et. al.

Ho et. al. recite many advantages of the overlap extension technique in page 58 and go on to state on page 59 "Gene splicing by overlap extension eliminates the need for the introduction of restriction sites and is ***independent of the targeted sequences***."

Zhang et. al. on page 4504 state "When coupled with the effective selection and applied reiteratively, such that the output of one cycle is the input for the next cycle, reiterative DNA shuffling has been demonstrated to be an efficient process for directed molecular evolution"

Thus Ho et. al. teaches a method that is *independent of the targeted sequences* i.e. they provide a tool where any sequence that is desirable can be created and need not be available to serve as a starting template. In this process of creating a desired

nucleotide sequence, it would be obvious to one of ordinary skill in the art that once a single molecule containing a desired element had been created then applying the method of Zhang et. al. it could be used a template to which the next addition of desirable feature could be added. Since the method of Zhang et. al. is efficient then one would have an efficient process to created desired molecules using this reiterative process.

13. Claims 5-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Horton et. al. (1989) Gene 77:61-68 (cited in previous Office Action) as evidenced by Ho et. al. (1989) Gene 77 51-59 (cited by applicant in IDS) in view of Zhang et. al. (1997) Proc. Natl. Acad. Sci. USA Vol. 94, pp 4504-4509 as applied to claim 1 above further in view of Haas et. al.(1996) Current Biology Vol. 6 No. 3 : 315-324.

A) Regarding claim 5, Horton et. al. & Zhang et. al. teaches the method of synthesizing target nucleotide according to claim 1.

But regarding claim 5 Horton et. al. & Zhang et. al. do not teach:

Further comprising determining if the target polynucleotide sequence is heterogeneous to a host to be used in expressing the protein encoding the target polynucleotide, and adjusting a sequence of one or more sets of primer pairs to change a codon of the target polynucleotide to a codon which has a high expression efficiency in translating a corresponding amino acid in a cell of the host.

B.) Regarding claim 5, Haas et. al. teaches determining if the target polynucleotide sequence is heterogeneous to a host to be used in expressing the

protein encoding the target polynucleotide, and adjusting a sequence of one or more sets of primer pairs to change a codon of the target polynucleotide to a codon which has a high expression efficiency in translating a corresponding amino acid in a cell of the host (see page 316 Fig. 1 and par. 1-3 where adjusting a sequence of one or more sets of primer pairs to change codons of the target polynucleotide (jelly fish GFP) to codons which has a high expression efficiency in translating a corresponding amino acid in a cell of the host (mammalian cells) is taught. See page 318 par. 3 where codon replacement results in enhancement of GFP expression is taught).

Regarding claim 6, Haas et. al. teaches wherein the host is an enteric bacterium (see page 321 par. 2 where enteric bacterium host *Escherichia coli* is taught).

C) It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to use the method taught by Haas et. al. in the method taught by Horton et. al. & Zhang et. al. The motivation to do so is provided by Haas et. al. who state "Codon bias has been observed in many species and a correlation has been noted between high expression and the use of a stereotyped pattern of codons.-------in several cases it has been found that expression of exogenous gene products in *Escherichia coli* can be enhanced by systematic substitution of the endogenous codons with triplets over-represented in highly expressed *E. coli* genes." (see page 321 par. 2). Thus by changing the codons of the target sequence to the codon bias of the intended host one of ordinary skill in the art would enhance expression of their target in the intended expression host.

14. Claims 7-9 and 12-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Horton et. al. as evidenced by Ho et. al. & Zhang et. al. as applied to claims 1-4 above, and Horton et. al. as evidenced by Ho et. al. & Zhang et. al. and Haas et. al. as applied to claims 5 and 6 above further in view of Baneyx F. (1999) Curr. Opin. Biotechnol. 10(5): 411-421 (cited in previous Office Action).

A) Regarding claim 7, Horton et. al. & Zhang et. al. teaches a method of producing the target polynucleotide according to claim 1.

Regarding claim 12, Horton et. al. & Zhang et. al. teaches a method of producing the target polynucleotide according to claim 2.

Regarding claim 13, Horton et. al. & Zhang et. al. teaches a method of producing the target polynucleotide according to claim 3.

Regarding claim 14, Horton et. al. & Zhang et. al. teaches a method of producing the target polynucleotide according to claim 4.

Regarding claim 15, Horton et. al. & Zhang et. al. teaches a method of producing the target polynucleotide according to claim 5.

Regarding claim 9, Horton et. al. & Zhang et. al. and Haas et. al. teaches a method according to claim 8 which further comprises, adjusting a sequence of one or more sets of primer pairs to change the fragments of the target polynucleotide used for expressing the target heterogeneous polypeptide, by changing the codon CTA encoding leucine to CTG, CTT, CTC, TTG, or TTA; the codon ATA encoding isoleucine to ATC or ATT; the codons CGG, AGG, AGA encoding arginine to CGT or CGC; the codon GGA encoding glycine to GGT or GGC; the codon CCC encoding proline to CCG, CCA or

CCT; the codon CTA encoding leucine to CTG, CTT, CTC, TTG, or TTA; the codon ATA encoding isoleucine to ATC or ATT; the codons CGG, AGG, AGA encoding arginine to CGT or CGC; the codon GGA encoding glycine to GGT or GGC; or the codon CCC encoding proline to CCG, CCA or CCT (see Haas et. al. page 321 par. 2 where codons used by highly expressed genes of *E.coli* are taught. One of ordinary skill in the art can look up the *E. coli* codon usage bias table and discern that the changes recited above are preferred codons for those amino acids in E.coli. Thus Haas et. al. teaches changing the codons as recited in claim 9 above)

B) But neither Horton et. al. & Zhang et. al. nor Horton et. al.; Zhang et. al. and Haas et. al. teaches :transforming a host or teaches expressing the target protein in the transformed host.

Regarding claims 7, Baneyx (1999) teaches: A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of: (2) transforming the target polynucleotide to the host; and (3) expressing the target heterogeneous protein in the transformed host. (see Baneyx page 413, par. 1-3 where method for transforming host and highly expressing a target polypeptide are taught).

Regarding claim 8 Baneyx teaches wherein the host is an enteric bacterium (see page 413, par. 1 where enteric bacterium *E.coli* is taught).

Regarding claims 12-15, Baneyx teaches method for transforming production host and highly expressing a target polypeptide (see page 413, par. 1-3).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to use method of Baneyx in method of Horton et. al. & Zhang et. al. or the method of Horton et. al.; Zhang et. al. and Haas et. al with a reasonable expectation of success.

The motivation to do so is provided by the review article of Baneyx who states" Among the many systems available for heterologous protein production, the Gram-negative bacterium *Escherichia coli* remains one of the most attractive because of its abilities to grow rapidly and at high density on inexpensive substrates, its well characterized genetics and the availability of an increasingly large number of cloning vectors and mutant host strains." (Baneyx page 411, par.1 Introduction).

15. Claim 19 is rejected under 35 U.S.C. 103(a) as being unpatentable over Horton et. al. (1989) Gene 77:61-68 as evidenced by Ho et. al. (1989) Gene 77 51-59 in view of Zhang et. al. (1997) Proc. Natl. Acad. Sci. USA Vol. 94, pp 4504-4509 as applied to claim 1 above, and further in view of Stemmer et. al. (US Pat. 5834252 issued November 10, 1998).

Regarding claim 19, Horton et. al. and Zhang et. al. teach the method of claim 1. But Horton et. al. and Zhang et. al. do not teach wherein each of the multicyclic polymerase chain reactions is conducted only on a template that consists of a product that has been extended with the addition of nucleotides in a previous polymerase chain reaction whereby the product of each succeeding polymerase chain reaction is longer than the product of each previous polymerase chain reaction.

Regarding claim 19, Stemmer et. al. teaches : wherein each of the multicyclic polymerase chain reactions is conducted only on a template that consists of a product that has been extended with the addition of nucleotides in a previous polymerase chain reaction whereby the product of each succeeding polymerase chain reaction is longer than the product of each previous polymerase chain reaction.(see col. 3 lines 32-64 and Fig. 1 A where initial DNA (step 1) is extended by addition of nucleotides in a PCR reaction (step 3). This extended product is further extended as shown in (steps iii or iv) depending on type of primer used. the process can be repeated and will result in product of each succeeding polymerase chain reaction is longer than the product of each previous polymerase chain reaction. The idea is further illustrated in Fig. 1B.

It would have been *prima facie* obvious to one of ordinary skill in the art to practice the method of Stemmer et. al. in the method of Horton et. al. & Zhang et. al. at the time of the invention. The motivation to do so is provided by Stemmer et. al. (see col. 1 lines 15- col. 2 lines 1- 62) who after describing the drawbacks of the existing PCR amplification including the limitations of method taught by Horton et. al. in col. 1 lines 54-67 goes on to state "In particular, there is a need for a PCR amplification method which can be performed with (1) only a single primer species, or (2) with multiple overlapping polynucleotides fragments (or oligonucleotides) in the absence of a conventional PCR primer".

One of ordinary skill in the art would be assured that there was a reasonable expectation of success if the method of Stemmer et. al was practiced in the method of Horton et. al. and Zhang et. al. since Stemmer et. al also used two part primers (see

Stemmer et. al. (col. 3, lines 32-42) as recited in claim 1. So now one would have a method where any desired polynucleotide could be made using bivalent primers such that multiple overlapping oligonucleotide primers could be used to successively extend the PCR product templates of the previous PCR reaction in a reiterative manner till desired product was obtained.

Conclusion

16. All claims 1-19 are rejected. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Suchira Pande whose telephone number is 571-272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Suchira Pande
Examiner
Art Unit 1637

TERESA E. STRZELECKA, PH.D.
PRIMARY EXAMINER

Teresa Strzelecka
10/30/06